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Therapy of rheumatoid arthritis by blocking IL-6 signal transduction with a humanized anti-IL-6 receptor antibody

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Introduction

The pathogenesis of autoimmune disease comprises several stages; (1) sensitization phase, (2) autoimmune phase, (3) chronic inflammatory phase, and (4) organ destruction phase as shown in Fig. 1. The etiology of rheumatoid arthritis (RA) is still unclear, but probably involves two factors, the major histocompatibility antigens (MHC) as intrinsic factors, and extrinsic factors, possibly antigens of bacteria, viruses or other micro-organisms. Specific T cells activated with antigen/MHC complex on the antigen-presenting cells may cross-react with autoantigens. From the pathological point of view, chronic inflammation is observed in the affected tissues in which immunocompetent cells proliferate, differentiate and produce chemical mediators (cytokines and immunoglobulins) [51]. Therapeutic methods aimed at blocking each of these steps are being developed. Practically, blocking cytokine function in the chronic inflammatory phase is one of the most acceptable methods, because it is now known that de-regulated cytokine production plays a major role in the pathogenesis of chronic inflammatory autoimmune diseases. Interleukin-6 (IL-6) is one of the principal inflammatory cytokines. In this review, the role of IL-6 in the pathogenesis of RA is discussed, and new therapy blocking IL-6 signal transduction with humanized anti-IL-6 receptor antibody is introduced.

Pleiotropic functions of IL-6

IL-6 was originally identified as an antigen-nonspecific B cell differentiation factor (BCDF) produced by activated mononuclear cells [69]. BCDF/B cell stimulation factor-2(BSF-2)/IL-6 induces the final maturation of B cells into antibody-forming cells

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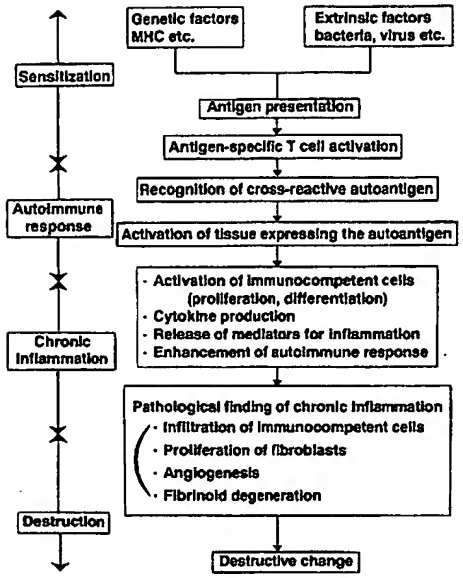

Fig. 1. Representation of etiology and pathogenesis in autoimmune disease

[18]. Following the determination of the nucleotide and amino acid sequences of BCDF/BSF-2/IL-6 [19], IL-6 was shown to be a pleiotropic cytokine with a wide range of biological activities. Figure 2 summarizes the many functions of IL-6.

In the immune response, IL-6 induces differentiation of B cells into antibody-forming cells [69], thus increasing the production of polyclonal immunoglobulins [18]. Another function of IL-6 is to promote IL-2 production in activated T cells [12]. Together with the induction of IL-2 receptor (IL-2R) expression on T cells, IL-6 can induce both the growth of T cells and the differentiation of T cells into cytotoxic T cells in the presence of IL-2 [7, 31, 33, 40, 46, 55]. The function of IL-6 is not restricted to the immune response, as it also acts on hematopoiesis [23, 29, 32, 44, 52], thrombocytosis [17, 24, 25, 30], inflammatory phenomenon [5, 38, 39, 45], and on the growth activity of various kinds of cells [14, 15, 21, 27, 28, 41, 62, 71].

To determine whether the reported in vitro functions of IL-6 occur in vivo, human IL-6 transgenic mice (C57BL/6) were generated by introducing the human IL-6 genomic gene fused with the human immunoglobulin heavy chain enhancer (Eμ) [53]. In IL-6 transgenic mice, IL-6 was constitutively produced by B cells; serum IL-6 was elevated. A polyclonal hypergammaglobulinemia was observed with plasmacytosis in the spleen and lymph nodes, and with an infiltration of plasma cells in liver, kidney and lung. In these mice there was also an increase of megakaryocytes in the bone marrow, and mesangial proliferative glomerulonephritis in the kidney [53]. In 1989, we demonstrated that the dysregulated production of IL-6 caused the main pathogenesis of plasma cell type of Castleman's disease [70]. These data indicate that IL-6 has the same functions in vivo as in vitro.

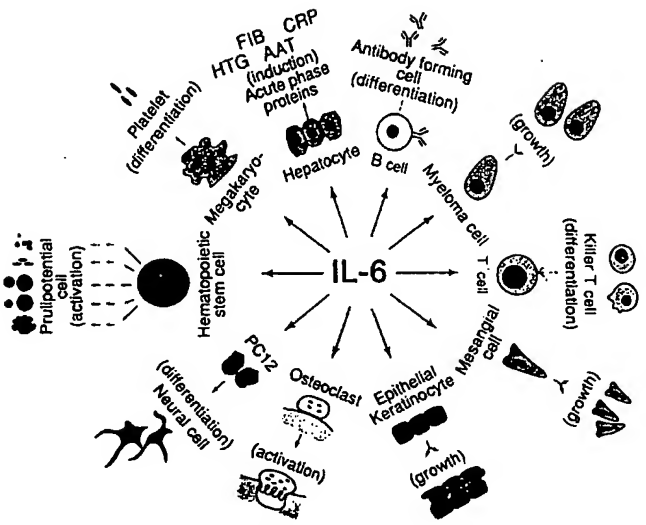

Fig. 2. Pleiotropic function of interleukin (IL-6)

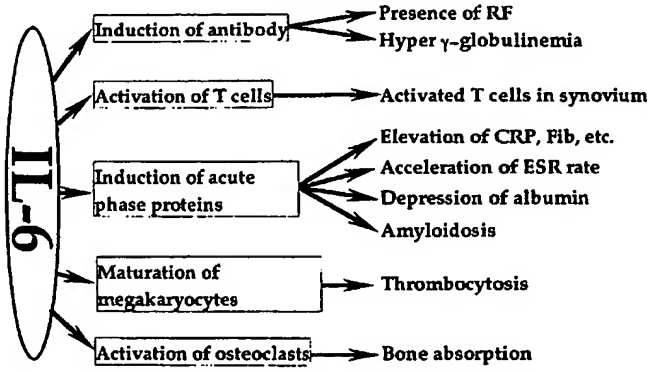

Fig. 3. The clinical abnormalities of rheumatoid arthritis (RA) can be explained by hyperproduction of IL-6 (*RF* rheumatoid factor)

IL-6 in RA

RA is a chronic inflammatory disease characterized by persistent synovitis and progressive destruction of cartilage and bone with the presence of an anti-immunoglobulin autoantibody, rheumatoid factor. RA is also associated with increases in erythrocyte sedimentation rate (ESR), elevation of acute-phase proteins, thrombocytosis, anemia, hypoalbuminemia, and polyclonal hypergammaglobulinemia besides local inflamma-

tion at multiple joints. Once the pleiotropic functions of IL-6 were recognized (Fig. 2), most of the clinical abnormalities seen in RA could be explained by the dysregulated hyperproduction of IL-6. As shown in Fig. 3, hyperproduction of IL-6 may induce an elevation of serum levels of polyclonal gammaglobulins and the presence of autoantibodies as a result of B cell differentiation and activation of autoreactive T cells. T cells activated by IL-6 and antigens, such as collagen and/or heat shock protein, may induce several cytokines as well as cytotoxic T cells through the induction of IL-2R on T cells. IL-6, as a hepatocyte-stimulating factor (HSF), may also induce acute-phase proteins, resulting in an elevation of serum levels of fibrinogen, C-reactive protein (CRP), haptoglobin, ceruloplasmin, α2-macroglobulin, α1-acid glycoprotein and amyloid protein, and a decrease in serum albumin [1, 5, 13, 26]. Furthermore, hyperproduction of IL-6 may cause bone absorption, resulting in osteoporosis and bone destruction through activation of osteoclasts [57]. Finally, IL-6 may induce thrombocytosis by acting as a differentiation factor of megakaryocytes to produce platelets [24, 25]. In addition to the abnormal laboratory findings, some of the symptoms of RA may also be related to the deregulated IL-6 production [34]. Patients with RA frequently complain of general fatigue, low appetite, loss of weight, and a subfebrile state, which may be explained by elevation of IL-6 in their serum.

Elevation of IL-6 levels was observed in both serum and synovial fluid in the patients with RA [20, 22, 48]. IL-6 is produced by activated macrophages, synovial cells and lymphocytes in affected joints and enlarged lymph nodes [43], and may be involved in the pathogenesis of pannus formation, angiogenesis, and destruction of cartilage and bone. Therefore, it may be possible to apply a specific therapy to patients with RA consisting of the suppression of IL-6 production, or inhibition of IL-6 function.

Blocking IL-6 signal transduction as a therapeutic method

Since conventional therapy with non-steroid anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs DMARDs combined with methotrexate (MTX) and/or steroids in RA is still unsatisfactory, new therapeutic strategies need to be defined. On the basis of the abnormal clinical and laboratory findings in RA which may be explained by hyperproduction of IL-6 mainly in the joints, interference with IL-6 signal transduction may constitute a new therapeutic strategy. It is known that the IL-6 signal is mediated via the IL-6 receptor (IL-6R) molecule (80 kDa) on the affected cells [67], followed by dimerization of associated signal transducer, gp130 (130 kDa), which is bound to the IL-6/IL-6R complex [16, 37].

Therefore, several therapeutic approaches can be proposed which interfere with the IL-6 signal transduction pathway (shown in Figure 4): (1) neutralization of IL-6; (2) blockade of IL-6 binding on IL-6R; (3) blockade of IL-6/IL-6R complex binding to gp130; (4) suppression of IL-6R and/or gp130 expression; and (5) blockade of the intracytoplasmic signal through gp130.

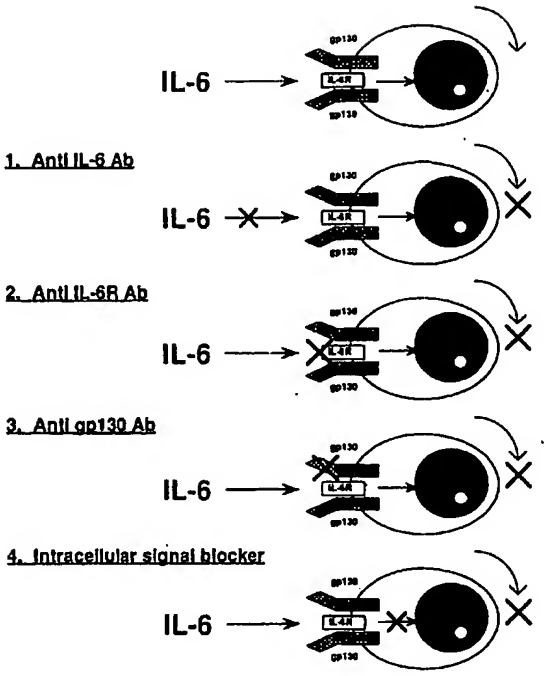

Fig. 4. RA therapy by blocking IL-6 signal transduction

Prevention of murine arthritis by blocking IL-6 signal with anti-IL-6R antibody

Collagen-induced arthritis (CIA) is an experimental arthritis model which is widely used for analyzing the pathogenesis of human RA [8, 60, 61]. CIA is also used for evaluating potential therapies for human RA. Previous reports have indicated that inflammatory cytokines, such as IL-1 [63] and tumor necrosis factor-α (TNF-α) [65], play roles in the pathogenesis of CIA. Sugita et al. and Takai et al. [54, 56] suggested the pathogenic involvement of IL-6 in CIA in which serum levels of IL-6 were elevated. However, no direct evidence for IL-6 involvement was proven in the development of CIA.

To confirm a pathogenic role of IL-6 in CIA, we have attempted to suppress the development of arthritis using the rat monoclonal anti-mouse IL-6R antibody, MR16-1. MR16-1 (0.5–8 mg) administered every day for 2 weeks to DBA/1J mice which had been immunized with bovine type II collagen. The clinical symptoms of arthritis in the four limbs were evaluated with a visual scoring system. Arthritic lesions were graded on a scale of 0–4 in one limb. MR16-1 injected on days 0 and 3 after immunization with type II collagen suppressed the development of arthritis in a dose-dependent manner as shown in Fig. 5.

A previous study reported that treatment of CIA with anti-TNF-α and anti-IL-1 antibodies had a clinical effect on established arthritis [63, 65]. However, anti-IL-6R an-

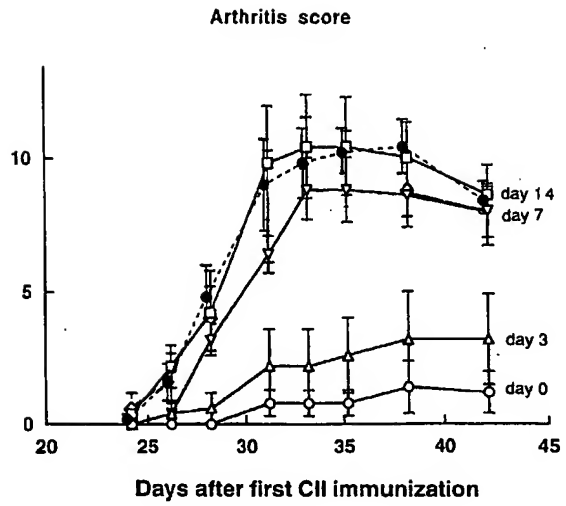

Fig. 5. Prevention of murine collagen-induced arthritis (CIA) by administration of rat anti-mouse IL-6 receptor antibody, MR16-1. MR16-1 (8 mg) was administered to DBA/1J mice immunized with bovine type II collagen (CII). MR16-1 was injected on day 0 (○), day 3 (△), day 7 (▽) and day 14 (square) after the first C II immunization. As a control, MR16-1 was not administered *(closed circles)*. The clinical symptoms of arthritis in all four limbs were evaluated with a visual scoring system. Arthritic lesions were graded on a scale of 0–4: 0, no change; 0.5, swelling and erythema of one digit; 1, swelling and erythema of two or more digits; 2, mild swelling and erythema of the limb; 3, gross deformity and inability to use the limb. The arthritis score for each mouse was the sum of the score of each of the four limbs

tibody was effective only in the early phase, and not on established arthritis, suggesting that the function of IL-6 might be different from that of TNF-α or IL-1. These results confirm that IL-6 plays an essential role in the pathogenesis of CIA, and that the blocking of IL-6 signal with anti-IL-6R antibody may be a useful approach for the treatment of CIA.

Humanized anti-IL-6R antibody

Wendling et al. [64] reported that the administration of mouse monoclonal anti-IL-6 antibody to patients with RA led to a partial and apparent improvement in the RA symptoms. However, the effect of the antibody treatment might be transient because the antibodies to mouse immunoglobulin were induced in the patients after repeated injection of mouse monoclonal antibody which could cause a functional reduction of anti-IL-6 antibody effect. Moreover, the antibody treatment can easily induce an allergic reaction. To prevent the induction of antibodies to mouse immunoglobulin in patients, remodelled human anti-IL-6R antibody(rhPM-1) was generated from mouse monoclonal anti-IL-6R antibody(PM-1) in CHO cells which were transfected with reshaped human γ_1-immunoglobulin gene inserted mouse CDR region of PM-1 as shown in Fig. 6. The binding capacity to IL-6R and the inhibitory effect of IL-6 function of the original PM-1 were conserved in the protein molecule of rhPM-1 [50]. rhPM-1 induced

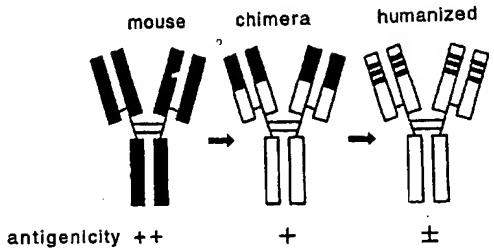

Fig. 6. Reshaped human anti-IL-6R antibody (rhPM-1) was produced from the mouse monoclonal antibody to human IL-6 receptor (PM-1) in CHO cells which were transfected with reshaped human γ immunoglobulin gene inserted mouse CDR region of PM-1. Antigenicity of humanized antibody is reduced compared to that of mouse antibody or chimeric antibody in human

hardly any idiotypic antibody and can thus be repeatedly injected and is, in principle, safer than both mouse or chimeric antibody in terms of allergic reaction induction.

Therapeutic approach with humanized anti-IL-6R antibody, rhPM-1

Inhibition by rhPM-1 of the development of experimental arthritis in monkeys

Experiments on arthritis in non-human primates are essential since they may demonstrate similar physiological response and immunological features to those of human arthritis because of their close phylogenetic relationship [2]. Moreover, the results of experiments concerning immunomodulating therapy for human disease performed in monkeys have a greater predictive value for human clinical results than experiments done in rodents, because the antigenicity of immunomodulators, such as monoclonal antibodies, may be common to humans and monkeys.

Since it has been shown that rat anti-mouse IL-6R antibody prevents the development of CIA in DBA/1J mice, and that CIA can be induced in non-human primates such as cynomolgus and rhesus monkeys [3, 6, 47, 58, 68], the therapeutic effect of rhPM-1 on arthritis can be examined using the model of cynomolgus monkey CIA.

Cynomolgus monkeys *(Macaca fascicularis)* were immunized with bovine type II collagen and boosted after 4 weeks. The clinical symptoms of arthritis appeared 4 weeks after the first immunization and increased up to 6–8 weeks in number and degree of swelling joints, stiff joints and edema of limbs. After 6–8 weeks arthritis quickly diminished. In the preliminary experiment [36], administration of rhPM-1 once a week for 13 weeks dose-dependently inhibited the onset of arthritis. The increase in the number of stiff joints and edema of limbs was suppressed in four out of five monkeys at a dose of 10 mg/kg rhPM-1 (Fig. 7). For swollen joints, the effect of 10 mg/kg rhPM-1 was not so clear. At the end of the experiment (14 weeks), all monkeys were killed and subjected to histological examination. In the control group without rhPM-1, most of the pathological changes in affected joints seemed similar to those of human RA, with synovial proliferation, pannus formation, infiltration of neutrophils, angiogenesis, and cartilage and bone destruction. These changes were primarily observed in the small joints of the hands and feet. Conversely, in the group treated with rhPM-1,

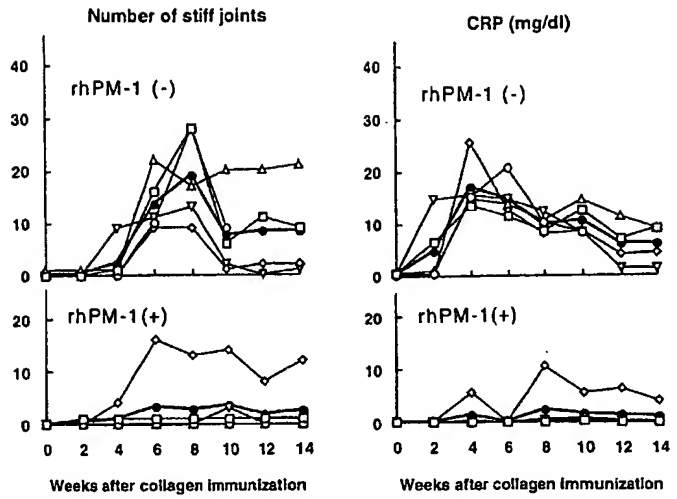

Fig. 7. Clinical course of symptomatic and laboratory findings in cynomolgus monkey CIA treated with rhPM-1. Stiffness of joints and serum levels of C-reactive protein (CRP) are represented as symptomatic and laboratory findings, respectively. rhPM-1 (10 mg/kg) was injected intravenously once a week for 13 weeks starting on the same day as the immunization with bovine type II collagen. Administration of rhPM-1 reduced the stiffness of joints and the serum levels of CRP as shown in the lower figures

these pathological changes were not seen, clearly demonstrating that rhPM-1 prevented the onset of CIA in cynomolgus monkeys.

In addition, administration of rhPM-1 to the cynomolgus monkeys did not affect other organs. Functions of liver, kidney and heart were normal, and the numbers of RBC, WBC and platelets remained within normal ranges. No symptomatic or functional side effects were observed. Taken together, these findings indicate that rhPM-1 seems to be a useful and effective reagent for the treatment of human RA.

Treatment of RA with rhPM-1

To evaluate the therapeutic effects of rhPM-1, which has the advantage of being less immunogenic for humans than mouse or chimeric monoclonal antibodies, we treated patients with severe RA who were resistant to any conventional therapy. These patients were suffering from continuous arthralgia with or without joint deformity, swollen joints and morning stiffness, combined with systemic symptoms of general fatigue, low appetite, loss of weight and subfever, despite treatment with NSAIDs, DMARDs, MTX and maintenance doses of steroids. The patients treated with rhPM-1 had more than six of the eight following criteria: (1) ESR > 40 mm; (2) morning stiffness for more than 60 min; (3) joint pain and/or swelling affecting more than 10 joints; (4) CRP greater than 3.0 mg/100 ml; (5) anemia due to chronic inflammation with less than 10 mg/100 ml Hb; (6) ferritin concentration greater than 100 mg/100 ml; (7) more than 35 $\times 10^4$ platelets/mm^3; and (8) serum IL-6 of more than 10 pg/ml.

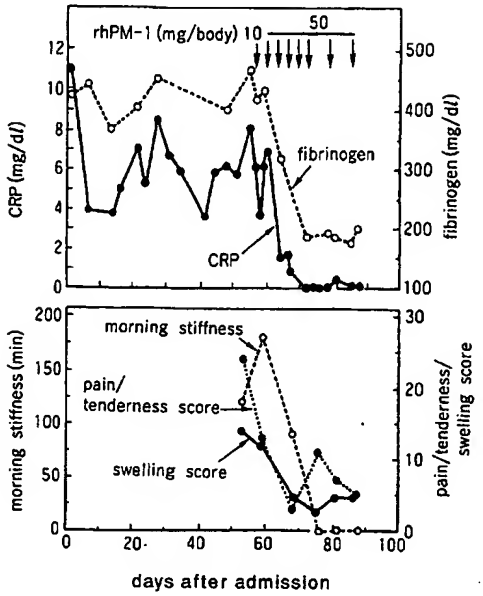

Fig. 8. Clinical course of laboratory and symptomatic findings in a patient with severe RA treated with rhPM-1. A 67 year-old woman with severe RA given NSAIDs, DMARDs, MTX and 15 mg predomizolone received 50 mg rhPM-1 twice a week or once a week combined with the conventional treatment. The clinical and laboratory abnormalities improved after the rhPM-1 therapy

After obtaining permission from the medical ethics committee of Osaka University for the treatment of severe RA with rhPM-1, and informed consent from the patients, treatment of RA with rhPM-1 was started. rhPM-1, 1-50 mg in 50 ml of saline was intravenously injected once or twice a week. The results were positive in all of the patients. From the findings obtained from the rhPM-1 treated patients, low-grade fever and fatigue disappeared within a week of starting rhPM-1 treatment. Serum CRP and fibrinogen levels were normalized within 2 weeks. Morning stiffness, swollen joint score, pain and tenderness score as well as anemia, thrombocytosis, hypoalbuminemia, and polyclonal hypergammaglobulinemia improved. The representative clinical course of a rhPM-1 treated patient is shown in Figure 8. These therapeutic effects did not decrease even after continuous 6-months treatment, in which the maintenance dose was 50 mg of rhPM-1 and the total amount was almost 1.2–2.4 g. No major side effects were observed except for the appearance of anti-idiotypic antibody in one case. The results of this open study suggest that rhPM-1 is effective, safe and useful for the treatment of RA, and that IL-6 is a pathogenic key cytokine as an effector in RA.

Conclusions

Recent molecular and genetic studies have analyzed the pathogenic mechanism of RA. Based on this pathogenic evidence, new therapeutic strategies are proposed in the field

of RA. Regulation of cytokine production and functions is one promising strategy for RA therapy. At present, several trials are planned, for instance on neutralization or interference of TNF-α with specific antibody [9–11] and soluble TNF-α receptors [49, 66], blocking IL-1 function with IL-1R antagonist [4, 42], inhibition of NF-κB [59], and direct use of the anti-inflammatory cytokine IL-10 [35], as described in other chapters in this issue. Interference of IL-6 signal transduction with the humanized anti-IL-6R antibody, rhPM-1, is one of the promising therapeutic strategies described in this review.

We have shown the improvement in symptomatic and laboratory findings obtained after 6 months treatment with rhPM-1. Since IL-6 activates osteoclasts to induce bone absorption [57], we expect the clinical effects against osteoporosis and bone destruction with longer term rhPM-1 treatment will be used for advanced stages of RA.

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